



Sunesis-0021.ST25.txt
SEQUENCE LISTING

<110> Ballinger, Marcus
<120> Constructs for Homogenously Processed Preparations of Beta Site
App-Cleaving Enzyme
<130> 2004345-0021
<140> 10/726,967
<141> 2003-12-02
<160> 110
<170> PatentIn version 3.2
<210> 1
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Met Ala Gln Ala Leu Pro Trp Leu Leu Leu Trp Met Gly Ala Gly Val
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Leu Pro Ala His Gly Thr Gln His Gly Ile Arg Leu Pro Leu Arg Ser
20 25 30

Gly Leu Gly Gly Ala Pro Leu Gly Leu Arg Leu Pro Arg Glu Thr Asp
35 40 45

Glu Glu Pro Glu Glu Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val
50 55 60

Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr
65 70 75 80

Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser
85 90 95

Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr
100 105 110

Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val
115 120 125

Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp
130 135 140

Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile
Page 1

145		150		155		160
Ala Ala Ile Thr	Glu 165	Ser Asp Lys Phe	Phe 170	Ile Asn Gly Ser	Asn 175	Trp
Glu Gly Ile	Leu 180	Gly Leu Ala Tyr	Ala 185	Glu Ile Ala Arg	Pro 190	Asp Asp
Ser Leu	Glu 195	Pro Phe Phe Asp	Ser 200	Leu Val Lys	Gln 205	Thr His Val Pro
Asn	Leu 210	Phe Ser Leu	Gln 215	Leu Cys Gly	Ala Gly	Phe 220 Pro Leu Asn Gln
Ser 225	Glu Val	Leu Ala	Ser 230	Val Gly Gly	Ser Met 235	Ile Ile Gly Gly Ile 240
Asp His Ser	Leu Tyr 245	Thr Gly Ser	Leu Trp 250	Tyr Thr Pro	Ile Arg 255	Arg
Glu Trp Tyr	Tyr 260	Glu Val Ile Ile	Val 265	Arg Val Glu	Ile Asn 270	Gly Gln
Asp Leu	Lys 275	Met Asp Cys	Lys Glu 280	Tyr Asn Tyr	Asp Lys 285	Ser Ile Val
Asp Ser	Gly 290	Thr Thr Asn	Leu 295	Arg Leu Pro	Lys 300	Val Phe Glu Ala
Ala 305	Val Lys Ser	Ile Lys 310	Ala Ala Ser	Ser Thr 315	Glu Lys Phe	Pro Asp 320
Gly Phe Trp	Leu Gly 325	Glu Gln Leu	Val Cys 330	Trp Gln Ala	Gly Thr 335	Thr
Pro Trp Asn	Ile 340	Phe Pro Val	Ile Ser 345	Leu Tyr Leu	Met Gly 350	Glu Val
Thr Asn	Gln 355	Ser Phe Arg	Ile Thr 360	Ile Leu Pro	Gln Gln 365	Tyr Leu Arg
Pro Val	Glu 370	Asp Val Ala	Thr 375	Ser Gln Asp	Asp Cys 380	Tyr Lys Phe Ala
Ile 385	Ser Gln Ser	Ser Thr 390	Gly Thr Val	Met Gly 395	Ala Val Ile	Met Glu 400

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Gly Phe Tyr Val Val Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe Ala
405 410 415

Val Ser Ala Cys His Val His Asp Glu Phe Arg Thr Ala Ala Val Glu
420 425 430

Gly Pro Phe Val Thr Leu Asp Met Glu Asp Cys Gly Tyr Asn Ile Pro
435 440 445

Gln Thr Asp Glu Ser Thr Leu Met Thr Ile Ala Tyr Val Met Ala Ala
450 455 460

Ile Cys Ala Leu Phe Met Leu Pro Leu Cys Leu Met Val Cys Gln Trp
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Arg Cys Leu Arg Cys Leu Arg Gln Gln His Asp Asp Phe Ala Asp Asp
485 490 495

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Ser Glu Val Asn Leu Asp Ala Glu Phe Arg
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 1 5

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Val Gln Xaa Asp Xaa Gly
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Val Gln Xaa Asp Xaa Ala
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Val Arg His Gln Ser
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Ile Asp Gly Arg
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Ile Glu Gly Arg
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<223> Residues 74-101 of human BACE1 preprosequence

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Gly Tyr Tyr Val Glu Met Thr Val Gly Ser Pro Pro Gln Thr Leu Asn
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Ile Leu Val Asp Thr Gly Ser Ser Asn Phe Ala Val
20 25

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<223> Residues 118-145 of human BACE1 preprosequence

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Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val Tyr Val Pro Tyr Thr
1 5 10 15

Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp Leu
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<223> Residues 165-185 of Human BACE1 Preprosequence isoforms A and B

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Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp Glu Gly Ile Leu
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Gly Leu Ala Tyr Ala
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<223> Residues 280-298 of human BACE1 preprosequence isoform A

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Glu Tyr Asn Tyr Asp Lys Ser Ile Val Asp Ser Gly Thr Thr Asn Leu
1 5 10 15

Arg Leu Pro

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Glu Leu Asn Leu Glu Thr Asp
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<210> 58
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1 5

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Glu Ile Asn Phe Ser Ala Ser Phe
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Glu Pro Gly Arg Arg Gly
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126-1362 of human BACE1

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gctgccccgg gagaccgacg aaga

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 cggagggtccc ggtatgtgct ggac 24

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 ccagaggcag ctgtccagca cata 24

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 tcccgccgga tgggtgtata ccag 24

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 gtacacaggc agtctctggt atacacc 27

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 in 5' direction

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 cggctgcccc tgcgcagcgg cctggggggc gccccctgg ggctgcggct gccccgggag 60

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 atgggcgcgg gagtgtgcc tgccacggc acccagcacg gcatccggct gcccctgcgc 60

<210> 73
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 nucleotides 126-1362 of human BACE1 in 5' direction

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<210> 74
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 cgccatgtg cgggagtgt gcctgcccac ggc 33

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36

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 starting at position 25

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Pro Leu Gly Leu Arg Leu Pro Arg Leu Val Pro Arg Gly Ser Glu Thr
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 35 40 45

Val Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met
 50 55 60

Thr Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly
 65 70 75 80

Ser Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu His Arg
 85 90 95

Tyr Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly
 100 105 110

Val Tyr Val Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr
 115 120 125

Asp Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn
 130 135 140

Ile Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn
 145 150 155 160

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Trp Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala Arg Pro Asp
165 170 175

Asp Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln Thr His Val
180 185 190

Pro Asn Leu Phe Ser Leu Gln Leu Cys Gly Ala Gly Phe Pro Leu Asn
195 200 205

Gln Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile Ile Gly Gly
210 215 220

Ile Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg
225 230 235 240

Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu Ile Asn Gly
245 250 255

Gln Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile
260 265 270

Val Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys Val Phe Glu
275 280 285

Ala Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro
290 295 300

Asp Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln Ala Gly Thr
305 310 315 320

Thr Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu Met Gly Glu
325 330 335

Val Thr Asn Gln Ser Phe Arg Ile Thr Ile Leu Pro Gln Gln Tyr Leu
340 345 350

Arg Pro Val Glu Asp Val Ala Thr Ser Gln Asp Asp Cys Tyr Lys Phe
355 360 365

Ala Ile Ser Gln Ser Ser Thr Gly Thr Val Met Gly Ala Val Ile Met
370 375 380

Glu Gly Phe Tyr Val Val Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe
385 390 395 400

Ala Val Ser Ala Cys His Val His Asp Glu Phe Arg Thr Ala Ala Val
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410

415

Glu Gly Pro Phe Val Thr Leu Asp Met Glu Asp Cys Gly Tyr Asn Ile
420 425 430

Pro Gln Thr Asp Glu Ser Thr
435

<210> 77
<211> 48
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide for insertion of thrombin cleavage site into
soluble human proBACE1

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<210> 78
<211> 433
<212> PRT
<213> Artificial

<220>
<223> Soluble human proBACE1 containing an engineered RLPL site
starting at position 21

<400> 78

Thr Gln His Gly Ile Arg Leu Pro Leu Arg Ser Gly Leu Gly Gly Ala
1 5 10 15

Pro Leu Gly Leu Arg Leu Pro Leu Glu Thr Asp Glu Glu Pro Glu Glu
20 25 30

Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val Asp Asn Leu Arg Gly
35 40 45

Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr Val Gly Ser Pro Pro
50 55 60

Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser Ser Asn Phe Ala Val
65 70 75 80

Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr Tyr Gln Arg Gln Leu
85 90 95

Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val Tyr Val Pro Tyr Thr
100 105 110

Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp Leu Val Ser Ile Pro
 115 120 125

His Gly Pro Asn Val Thr Val Arg Ala Asn Ile Ala Ala Ile Thr Glu
 130 135 140

Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp Glu Gly Ile Leu Gly
 145 150 155 160

Leu Ala Tyr Ala Glu Ile Ala Arg Pro Asp Asp Ser Leu Glu Pro Phe
 165 170 175

Phe Asp Ser Leu Val Lys Gln Thr His Val Pro Asn Leu Phe Ser Leu
 180 185 190

Gln Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala
 195 200 205

Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile Asp His Ser Leu Tyr
 210 215 220

Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu
 225 230 235 240

Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln Asp Leu Lys Met Asp
 245 250 255

Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val Asp Ser Gly Thr Thr
 260 265 270

Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala Ala Val Lys Ser Ile
 275 280 285

Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp Gly Phe Trp Leu Gly
 290 295 300

Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr Pro Trp Asn Ile Phe
 305 310 315 320

Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val Thr Asn Gln Ser Phe
 325 330 335

Arg Ile Thr Ile Leu Pro Gln Gln Tyr Leu Arg Pro Val Glu Asp Val
 340 345 350

Ala Thr Ser Gln Asp Asp Cys Tyr Lys Phe Ala Ile Ser Gln Ser Ser
 355 360 365

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Thr Gly Thr Val Met Gly Ala Val Ile Met Glu Gly Phe Tyr Val Val
370 375 380

Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe Ala Val Ser Ala Cys His
385 390 395 400

Val His Asp Glu Phe Arg Thr Ala Ala Val Glu Gly Pro Phe Val Thr
405 410 415

Leu Asp Met Glu Asp Cys Gly Tyr Asn Ile Pro Gln Thr Asp Glu Ser
420 425 430

Thr

<210> 79
<211> 7
<212> PRT
<213> Artificial

<220>
<223> RLPL site

<400> 79

Arg Leu Pro Leu Glu Thr Asp
1 5

<210> 80
<211> 42
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide to introduce RLPL site into soluble human
proBACE1

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42

<210> 81
<211> 433
<212> PRT
<213> Artificial

<220>
<223> soluble human proBACE1

<400> 81

Thr Gln His Gly Ile Arg Leu Pro Leu Arg Ser Gly Leu Gly Gly Ala
1 5 10 15

Pro Leu Gly Leu Glu Leu Asn Leu Glu Thr Asp Glu Glu Pro Glu Glu
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Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val Asp Asn Leu Arg Gly
35 40 45

Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr Val Gly Ser Pro Pro
50 55 60

Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser Ser Asn Phe Ala Val
65 70 75 80

Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr Tyr Gln Arg Gln Leu
85 90 95

Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val Tyr Val Pro Tyr Thr
100 105 110

Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp Leu Val Ser Ile Pro
115 120 125

His Gly Pro Asn Val Thr Val Arg Ala Asn Ile Ala Ala Ile Thr Glu
130 135 140

Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp Glu Gly Ile Leu Gly
145 150 155 160

Leu Ala Tyr Ala Glu Ile Ala Arg Pro Asp Asp Ser Leu Glu Pro Phe
165 170 175

Phe Asp Ser Leu Val Lys Gln Thr His Val Pro Asn Leu Phe Ser Leu
180 185 190

Gln Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala
195 200 205

Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile Asp His Ser Leu Tyr
210 215 220

Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu
225 230 235 240

Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln Asp Leu Lys Met Asp
245 250 255

Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val Asp Ser Gly Thr Thr
260 265 270

Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala Ala Val Lys Ser Ile
275 280 285

Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp Gly Phe Trp Leu Gly
290 295 300

Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr Pro Trp Asn Ile Phe
305 310 315 320

Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val Thr Asn Gln Ser Phe
325 330 335

Arg Ile Thr Ile Leu Pro Gln Gln Tyr Leu Arg Pro Val Glu Asp Val
340 345 350

Ala Thr Ser Gln Asp Asp Cys Tyr Lys Phe Ala Ile Ser Gln Ser Ser
355 360 365

Thr Gly Thr Val Met Gly Ala Val Ile Met Glu Gly Phe Tyr Val Val
370 375 380

Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe Ala Val Ser Ala Cys His
385 390 395 400

Val His Asp Glu Phe Arg Thr Ala Ala Val Glu Gly Pro Phe Val Thr
405 410 415

Leu Asp Met Glu Asp Cys Gly Tyr Asn Ile Pro Gln Thr Asp Glu Ser
420 425 430

Thr

<210> 82
<211> 42
<212> DNA
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proBACE1

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42

<210> 83
<211> 27
<212> DNA
<213> Artificial

<220>

<223> oligonucleotide to introduce EINL site into soluble human proBACE1

<400> 83
ggctctccagg ttgatttcca gacccag

27

<210> 84
<211> 433
<212> PRT
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<400> 84

Thr Gln His Gly Ile Arg Leu Pro Leu Arg Ser Gly Leu Gly Gly Ala
1 5 10 15

Pro Leu Gly Leu Glu Ile Asn Leu Glu Thr Asp Glu Glu Pro Glu Glu
20 25 30

Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val Asp Asn Leu Arg Gly
35 40 45

Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr Val Gly Ser Pro Pro
50 55 60

Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser Ser Asn Phe Ala Val
65 70 75 80

Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr Tyr Gln Arg Gln Leu
85 90 95

Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val Tyr Val Pro Tyr Thr
100 105 110

Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp Leu Val Ser Ile Pro
115 120 125

His Gly Pro Asn Val Thr Val Arg Ala Asn Ile Ala Ala Ile Thr Glu
130 135 140

Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp Glu Gly Ile Leu Gly
145 150 155 160

Leu Ala Tyr Ala Glu Ile Ala Arg Pro Asp Asp Ser Leu Glu Pro Phe
165 170 175

Phe Asp Ser Leu Val Lys Gln Thr His Val Pro Asn Leu Phe Ser Leu
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190

Gln Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala
 195 200 205

Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile Asp His Ser Leu Tyr
 210 215 220

Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu
 225 230 235 240

Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln Asp Leu Lys Met Asp
 245 250 255

Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val Asp Ser Gly Thr Thr
 260 265 270

Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala Ala Val Lys Ser Ile
 275 280 285

Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp Gly Phe Trp Leu Gly
 290 295 300

Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr Pro Trp Asn Ile Phe
 305 310 315 320

Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val Thr Asn Gln Ser Phe
 325 330 335

Arg Ile Thr Ile Leu Pro Gln Gln Tyr Leu Arg Pro Val Glu Asp Val
 340 345 350

Ala Thr Ser Gln Asp Asp Cys Tyr Lys Phe Ala Ile Ser Gln Ser Ser
 355 360 365

Thr Gly Thr Val Met Gly Ala Val Ile Met Glu Gly Phe Tyr Val Val
 370 375 380

Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe Ala Val Ser Ala Cys His
 385 390 395 400

Val His Asp Glu Phe Arg Thr Ala Ala Val Glu Gly Pro Phe Val Thr
 405 410 415

Leu Asp Met Glu Asp Cys Gly Tyr Asn Ile Pro Gln Thr Asp Glu Ser
 420 425 430

Thr

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<211> 27
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<210> 86
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<400> 86
actgctgcct gtacacacca ggatggt 27

<210> 87
<211> 27
<212> DNA
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<223> oligonucleotide for T133C mutation of human BACE1

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ttcccacttg ccacaggtgt agggcac 27

<210> 88
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<220>
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<400> 88
ttcccacttg ccacaggtgt agggcac 27

<210> 89
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<212> DNA
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<400> 89
gttgatgaag aaacagtctg attcagt 27

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<210> 90
 <211> 27
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 <220>
 <223> oligonucleotide for F169C mutation of human BACE1

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 <210> 91
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 gttggagccg ttacagaaga acttgtc 27

 <210> 92
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 <210> 93
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 <220>
 <223> oligonucleotide for I179C mutation of human BACE1

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 <210> 94
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 <210> 95
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<212> DNA
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<220>
 <223> oligonucleotide for R189C mutation of human BACE1

<400> 95
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<210> 96
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<210> 97
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<220>
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<210> 98
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<400> 98
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<210> 99
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<220>
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<400> 99
 ggtggtgcc ctacacacaa tgctctt 27

<210> 100
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<220>
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 <223> oligonucleotide for R296C mutation of human BACE1
 <400> 102
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 <210> 103
 <211> 27
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 <223> oligonucleotide for G325C mutation of human BACE1
 <400> 103
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 <210> 104
 <211> 27
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 <223> oligonucleotide for R368C mutation of human BACE1
 <400> 104
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 <210> 105
 <211> 27
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 <223> oligonucleotide for K382C mutation of human BACE1

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tgagatggca aaacagtaac agtcgtc 27

<210> 106
<211> 27
<212> DNA
<213> Artificial

<220>
<223> oligonucleotide for S386C mutation of human BACE1

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<210> 107
<211> 27
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<223> oligonucleotide for T390C mutation of human BACE1

<400> 107
cataacagtg ccacaggatg actgtga 27

<210> 108
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<220>
<223> oligonucleotide for V393C mutation of human BACE1

<400> 108
cagctcccat acaagtgccc gtggatg 27

<210> 109
<211> 27
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<220>
<223> oligonucleotide for inactivating D90N mutation of human BACE1

<400> 109
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<210> 110
<211> 27
<212> DNA
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<220>
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<400> 110
ggtggtgcca ctgttcacaa tgctctt 27

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